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Director of RWS Group Ltd, of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England declare;

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2. That the translator responsible for the attached translation is well acquainted with the French and English languages.

3. That the attached is, to the best of RWS Group Ltd knowledge and belief, a true translation into the English language of the accompanying copy of the specification filed with the application for a patent in The United States of America on 20 November 2002 under the number 60/427,575 and the official certificate attached hereto.

4. That I believe that all statements made herein of my own knowledge are true and that all statements made on information and belief are true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application in the United States of America or any patent issuing thereon.

For and on behalf of RWS Group Ltd

The 18th day of August 2005

PA 1328342

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APPLICATION NUMBER: ***60/427,575***
FILING DATE: ***November 20, 2002***

By Authority of the
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Docket Number	016800-556	Type a plus sign (+) inside this box	+

INVENTOR(S)/APPLICANT(S)

Last Name	First Name	Middle Initial	Residence (City and either State or Foreign Country)
NONE AT THIS TIME			

TITLE OF THE INVENTION (280 characters max)

USE OF A COMBINATION OF COMPONENTS WITH AN INHIBITORY SYNERGISTIC EFFECT ON CALCIUM CHANNELS TO PREVENT OR TREAT WRINKLES AND FINE LINES

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ENCLOSED APPLICATION PARTS *(check all that apply)*

[X] Specification *Number of Pages* 15 (French) [X] Drawing(s) *Number of Sheets* 1

[X] Other (specify) ABSTRACT OF THE DISCLOSURE, 17 CLAIMS - 2 PAGES, SEQUENCE LISTING - 2 PAGES (French)

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(CHECK ONE)*

[ ] Applicant claims small entity status. See 37 CFR § 1.27.	PROVISIONAL FILING FEE AMOUNT(S)	$ [ ] $80.00 (2005)
[X] A check or money order is enclosed to cover the Provisional filing fees.		$ [X] $160.00 (1005)
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.

[ ] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted.

(signature)

SIGNATURE _____ Date NOVEMBER 20, 2002

TYPED or PRINTED NAME NORMAN H. STEPNO Registration No. 22,716 *(if appropriate)*

[ ] Additional inventors are being named on separately numbered sheets attached hereto

(10/02)

The invention relates to a composition that is suitable for topical application to the skin, comprising, in a physiologically acceptable medium, (i) at least one peptide or a mixture of peptides comprising an amino acid sequence derived from the amino acid sequence of the protein SNAP 25, and (ii) at least one calcium-channel inhibitor.

Women, and even men, currently have a tendency to wish to look youthful for as long as possible and consequently seek to fade out the signs of aging on the skin, which are reflected in particular by wrinkles and fine lines. In this respect, the media and the fashion world report about products intended to keep the skin radiant and wrinkle-free for as long as possible, which are signs of youthful skin, and all the more so since the physical appearance acts on the psyche and/or on the morale.

Hitherto, wrinkles and fine lines were treated using cosmetic products containing active agents acting on the skin, for example by moisturizing it or by improving its cell renewal or alternatively by promoting the synthesis, or preventing the degeneration, of the elastic fibres which make up skin tissue.

Although these treatments make it possible to act on the wrinkles and fine lines caused by chronological or intrinsic ageing, and also on those

caused by photoageing, they have no effect on expression wrinkles, which require an intervention on the contractile muscle component of the wrinkles present in the skin.

Specifically, the Applicant has shown that the contractile muscle fibres, in particular striated muscle fibres, which are under the direct control of the neuromuscular impulse, play an essential role in the formation of expression wrinkles, and that modulating the neuromuscular impulse attenuates expression wrinkles and also has a "smoothing" effect on the skin's microrelief.

It is known that the dermal muscles of the face are under the control of motor nerve afferences of the facial nerve and that, moreover, the interlobular septa of the hypoderm contain within them fibres that constitute a striated muscle tissue. In the peripheral nervous system, the junction between a nerve and a striated muscle constitutes the neuromuscular plate, upstream of which is the afferent nerve pathway, known as the motor neuron. Moreover, cell membranes of each nerve fibre, and also of muscle cells, comprise numerous ion channels, and especially calcium channels, or chlorine channels, which are capable of allowing the controlled passage of Ca^{2+} or Cl^-, respectively.

Variations in the intracellular Ca^{2+} concentration are involved in initiating electrical and

mechanical phenomena, for example depolarization or contraction of smooth or striated muscle, hormonal secretion and activation of enzymes.

In particular, the increase in the calcium concentration is the cause of muscle contraction, and its decrease causes relaxation.

It is generally accepted that during the contraction phase, the thin actin filaments slide between the thick myosin filaments, thus resulting in shortening of the sarcomeres and consequently a contractile movement of the cell and of the fibre as a whole.

As regards skeletal striated muscle, in the relaxed state, the actin is not accessible to the myosin bridges because it is associated with another protein complex consisting of troponin and myosin.

When calcium binds to the troponin-myosin complex, the actin molecules become accessible and the contractile phenomenon can then begin. The troponin molecule undergoes a conformational change that reveals the ATPase activity of the heads of the myosin molecule in the thick filaments, thus initiating contraction. The hydrolysis of the ATP to ADP and phosphate provides the chemical energy allowing the filaments to slide.

The role of Ca^{2+} in the contractile proteins of striated muscle is thus an activating (de-inhibiting) role on the ATPase activity.

Relaxation of the striated muscle takes place when the transverse tubules and the cell membrane are repolarized, thus allowing the cellular intracytoplasmic Ca^{2+} concentration to return to a value of 10^{-7} M, below the activation threshold of intracellular enzymes such as ATPase (activation threshold which is in the region of 1 to 2 concentration logarithms higher).

In the contraction of smooth muscle fibre, calcium is not an activator *per se*: it combines with calmodulin, and the calcium-calmodulin complex activates MLCK (myosin light chain kinase), forming therewith a ternary complex. This complex converts the myosin into phosphorylated myosin, which combines with actin, resulting in a contraction of the smooth fibres.

The contraction-relaxation cycle is caused by variations in the cytoplasmic calcium concentration of from 10^{-7} M (inactive) to 10^{-5} M (active).

Regulating the intracellular cytoplasmic Ca^{2+} concentration is only possible because the cytoplasmic calcium effluxes correct the cytoplasmic influxes. The intracytoplasmic Ca^{2+} exchanges take place either with respect to intracellular storage vesicles or with respect to the exterior of the cell. In both cases, the Ca^{2+} is not available in the cytoplasm. These exchanges can be ensured only by expelling intracellular cytoplasmic calcium via one or more "active" mechanisms

capable of surmounting the electrochemical potential gradient mentioned previously.

Two types of mechanism can intervene: a calcium pump, which actively expels the cations at the expense of the hydrolysis of ATP, and a movement of Ca^{2+} coupled to a movement of Na^{+}. In most cells, the ATP-dependent calcium pump operates more efficiently in the presence of calmodulin, which increases its affinity.

In order better to describe the changes in permeability to calcium, it is currently common to consider that this permeability corresponds (i) to the opening of calcium channels that are dependent on the membrane potential, or VOCs (voltage-operated channels), which open during depolarization and allow calcium to enter the cell, or (ii) to the activation of membrane receptors.

Three types of VOC are mainly known: a channel that opens at a low potential, the T channel (Transient channel), and two types of channel that open at a high potential, the L channels (Long channels) and N channels (channels present in the neurons).

Moreover, it is very likely that these calcium channels show tissue specificity.

In 1965, T. Godfraind demonstrated that the permeability to calcium of the membrane might be inhibited by pharmacological agents, which would constitute the common mechanism on which Ca^{2+}

antagonists act.

It is thus understood from the text hereinabove that the contraction or hypercontraction of certain facial muscles, or of certain contractile cells of the dermis, for instance the myofibroblasts, may be induced by different mechanisms in particular involving Cl^-, Ca^{2+} and intracellular calcium ion exchanges, and that by acting in particular on the calcium channels, it is possible to relax these muscles or cells and thus smooth out expression wrinkles.

Hitherto, the means most commonly used for acting on expression wrinkles has been botulinum toxin, which is in particular injected into the wrinkles of the glabella, which are the wrinkles between the eyebrows (see J.D. Carruters et al., J. Dermatol. Surg. Oncol., 1992, 18, pp. 17-21). Dermatologists also make use of degradable implants based on collagen, hyaluronic acid or polylactic acid.

However, these methods have the drawback of requiring a medical intervention and do not give long-lasting results.

There is thus a need for effective compounds that can be used in a composition suitable for topical application to the skin for preventing or treating, especially for smoothing out or fading out wrinkles and fine lines, in particular expression wrinkles.

The Applicant has now discovered,

surprisingly, that:

1) certain peptides have the effect of inhibiting type-L calcium channels, and that this effect is observed on the three types of type-L calcium channels (DHP site, diltiazem site and verapamil site);

2) these peptides combined with magnesium have synergistic antagonist effects with respect to the type-L calcium channels, verapamil site.

These type-L calcium channels were identified in human fibroblasts (Baumgarten LB et al. (1992), *J. Biol. Chem.*, 267, 10524-10530 and Chen CF et al. (1988), *Science*, 239, 1024-1026). The DHP (dihydropyridine), diltiazem and verapamil sites correspond to the sites on the type-L calcium channels that are specific for the corresponding pharmacological agents known as calcium inhibitors. Furthermore, depending on its affinity for certain calcium channels and for certain tissues, each calcium-inhibiting pharmacological agent has a predominant effect corresponding to a preferential therapeutic indication.

Admittedly, patent application EP 1 180 524 discloses peptides with an anti-wrinkle effect, which, via a mechanism of inhibition of the SNARE complex (SNAP Receptor Complex), result in a reduction in the release of a neuromediator, acetylcholine, into the synaptic spaces.

The mechanism of action of these peptides is similar to that of botulinum toxins: they affect the formation and/or stability of the fusion protein complex (SNARE), which is a core of membrane proteins consisting of SNAP 25 (25 kDa Synaptosomal Associated Protein), syntaxin and synaptobrevin (or VAMP, for Vesicle-Associated Membrane Protein), the role of which is to mediate the neuronal exocytosis, i.e. the release of neurotransmitters (Ferrer Montier et al., 1997 *The Journal of Biological Chemistry*, 272, 2634-2638).

However, no inhibitory activity on calcium channels is described or suggested for these peptides. In addition, it is not suggested to combine them with a calcium-channel inhibitor.

Moreover, the role of calcium, and of regulating its intracellular concentration, in muscle contraction/relaxation phenomena are known. Moreover, it has previously been proposed to act on calcium channels in order to relax or decontract tissues, and thus reduce wrinkles and fine lines (FR-2 793 681).

However, no combination between (i) at least one peptide or mixture of peptides as defined below, and (ii) at least one calcium-channel inhibitor, has been proposed hitherto.

The Applicant has now demonstrated that such a combination makes it possible to neutralize the formation of the expression wrinkles of the face: it

neutralizes the effects of microtensions on the skin and thus makes it possible to fade out expression wrinkles and to prevent them from deepening, while still allowing facial expressions.

One subject of the present invention is thus a composition suitable for topical application to the skin, comprising, in a physiologically acceptable medium, (i) at least one peptide or mixture of peptides comprising an amino acid sequence derived from the amino acid sequence of the protein SNAP 25, and (ii) at least one calcium-channel inhibitor.

The term "sequence derived from the amino acid sequence of the protein SNAP 25" means any sequence or sequence fragment coding for SNAP 25, defined by SEQ ID No. 1 or any sequence that differs from the sequence SEQ ID No. 1 by mutation, insertion, deletion or substitution of one or more bases, or by degeneracy of the genetic code, provided that it codes for a polypeptide having the activity of SNAP 25.

This peptide may have an amino acid sequence comprising from 3 to 30 amino acids and preferably from 6 to 19 amino acids, in which the N-terminal amino acid may be acetylated and/or the C-terminal amino acid may be amidated.

A preferred peptide that may be used in the context of the invention is the hexapeptide defined by SEQ ID No. 2 (acetyl hexapeptide-3).

It is also possible to use a peptide chosen from:

(i) a peptide that is "substantially homologous" with the peptide defined by SEQ ID No. 2;

(ii) a peptide that is "functionally equivalent" to the peptide defined by SEQ ID No. 2;

(iii) a cosmetically acceptable salt of the said peptide defined by SEQ ID No. 2;

(iv) a peptide defined by SEQ ID No. 2 that has undergone reversible chemical changes.

The hexapeptide mentioned above is available from the company Lipotec under the trade name Argireline®: it contains a sequence of 6 amino acids: glutamyl- glutamyl- methionyl- glutaminyl- arginyl- arginyl, the first (N-terminal) being acetylated, and the last (C-terminal) being amidated.

The expression "substantially homologous" peptide or amino acid sequence means an amino acid sequence that is at least 60%, preferably at least 80% and even more preferably at least 95% identical to the sequence SEQ ID No. 2.

For the purposes of the present invention, the term "percentage of identity" between two amino acid sequences is intended to denote a percentage of amino acid residues that are identical between the two sequences to be compared, obtained after the best alignment, this percentage being purely statistical and

their differences between the two sequences being distributed randomly and over their entire length. The terms "best alignment" and "optimum alignment" are intended to denote the alignment for which the percentage of identity determined as below is the highest. The sequence comparisons between two amino acid sequences are conventionally performed by comparing these sequences after they have been optimally aligned, the said comparison being performed by segment or by "window of comparison" to identify and compare the local regions of sequence similarity. The optimum alignment of the sequences for the comparison may be performed, other than manually, by means of the Smith-Waterman local homology algorithm (1981, Ad. App. Math. 2: 482), by means of the Neddleman-Wunsch local homology algorithm (1970, J. Mol. Biol. 48: 443), by means of the Pearson-Lipman similarity search method (1988, Proc. Natl. Acad. Sci. USA 85: 2444) or by means of computer software using these algorithms (GAP, BESTFIT, BLAST P or BLAST N, available on the site NCBI, FASTA and TFASTA in Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr, Madison, WI). In order to obtain the optimum alignment, the BLAST program is preferably used, with the BLOSUM 62 matrix. The PAM or PAM2590 matrices may also be used.

The percentage of identity between two amino

acid sequences is determined by comparing these two optimally aligned sequences in which the amino acid sequence to be compared may comprise additions or deletions relative to the reference sequence for an optimum alignment between these two sequences. The percentage of identity is calculated by determining the number of identical positions for which the amino acid residue is identical between the two sequences, dividing this number of identical positions by the number of compared positions, and multiplying the result obtained by 100 to obtain the percentage of identity between these two sequences.

The expression "functionally equivalent peptide" means a peptide that has at least the capacity to inhibit type-L calcium channels.

The expression "cosmetically acceptable salt of the said peptide" means metal salts or salts formed by addition of suitable acids or bases, which may be obtained from a reaction with the peptides according to the invention, according to the methods known to those skilled in the art.

Organic salts of peptides that may be mentioned include peptide gluconate, peptide acetate, peptide citrate, peptide oleate and peptide oxalate.

Mineral salts of peptides that may be mentioned include peptide chloride, peptide borate, peptide sulphate and peptide carbonate.

As "reversible chemical modifications" of the said peptide so as to increase its bioavailability and its ease of passing through epithelial tissue without affecting its capacity to inhibit the type-L calcium channels, examples that may be mentioned include the esterification reaction of the carboxylate groups of the amino acids glutamic acid and aspartic acid with an acetylmethyl group, thus removing the negative charge from the amino acid and increasing its hydrophobicity.

These peptides may be obtained via conventional methods of chemical peptide synthesis or methods based on recombinant DNA technology, which are well known to those skilled in the art. For example, the solid-phase chemical synthesis method described by Pennington et al. (1994, *Peptide synthesis protocols*, Humana Press, Totowa) may be used.

It is also possible to use, in the context of the invention, any peptide or peptide mixture chosen from:

a) a peptide comprising a sequence of 3 to 30 amino acids contained in SEQ ID No. 1 (protein SNAP 25); it preferably consists of adjacent amino acids;

b) a peptide comprising a sequence of 6 to 19 amino acids derived from the N-terminus of the protein SNAP 25, chosen from the group formed by the peptides defined by SEQ ID No. 2 and

SEQ ID No. 3;

c) a peptide comprising a sequence of 6 to 19 amino acids derived from the C-terminus of the protein SNAP 25, chosen from the group formed by the peptides defined by SEQ ID No. 5 and SEQ ID No. 6;

d) a peptide mixture consisting of at least one peptide of 3 to 30 amino acids from those described in a), b) and c) and of at least one peptide of 3 to 30 amino acids contained in SEQ ID No. 4 ((COOH) peptide sequence);

e) a peptide mixture consisting of at least one peptide chosen from the group formed by the peptides defined by SEQ ID No. 2 and SEQ ID No. 3 (N-terminus) and of at least one peptide chosen from the group formed by the peptides defined by SEQ ID No. 5 and SEQ ID No. 6 (C-terminus).

According to the invention, these peptides will be combined with another calcium-channel inhibitor, the activity of which will be potentiated.

Two classes of active agents are known as calcium-channel inhibitors, respectively:

1) agents that are active on the plasma membrane, inhibiting the entry of calcium;

2) agents that are active inside the cell (releasing intracellular reserves of Ca^{2+}, or inhibiting the

formation of the Ca^{2+} calmodulin complex).

In order for a substance to be recognized as a calcium-channel inhibitor, also referred to in the text as a calcium antagonist, it must be able to reduce the intracellular calcium concentration or to reduce the binding of calcium to intracellular proteins, for instance calmodulin, as is especially described, for example, by Galizzi, J.P. *et al.*, J. Biol. Chem. 1987, 262 p. 6947 or Y. Okamiya *et al.*, Eur. J. Pharmacol. 1991, 205, p. 49 or J.A. Wagner *et al.*, J. Neurosci. 1988, 8, p. 3354 or H.R. Lee *et al.*, Life Sci. 1984, 35 p. 721 or Schoemaker H. and Lauger S. Eur. J. Pharmacol. 1985, 111, p. 273, or I.J. Reynolds *et al.*, J. Pharmacol. Exp. Ther. 1986, 237, p. 731.

A substance is acknowledged as being relaxing when it shows a relaxing effect on contracted muscle tissue and/or shows an inhibitory effect in an experimental model of nerve-muscle junction (motor plate) especially in the model described by W. Steinbrecher in: *Electrodes for stimulation and bioelectric potential recording*, Ed. Biomerstechnich, 1988, pages 96-98.

Preferably, according to the invention, agents which are active on the plasma membrane, which inhibit the entry of calcium or which complex calcium, for instance alverine and/or its organic or mineral salts, manganese and/or its organic or mineral salts or

magnesium and/or its salts, are used.

These compounds may be of natural or synthetic origin. The term "natural origin" means a compound in pure form or in solution, irrespective of its concentration in the said solution, which may be obtained according to various extraction processes from a natural product. The term "synthetic origin" means a compound in pure form or in solution at any concentration, obtained by chemical synthesis.

Alverine and/or its organic or mineral salts may thus be used.

As organic alverine salts that may be used according to the invention, mention may be made of alverine gluconate, alverine acetate, alverine citrate, alverine oleate and alverine oxalate.

Mineral alverine salts that may be mentioned include alverine chloride, alverine borate, alverine nitrate, alverine phosphate, alverine sulphate and alverine carbonate.

Preferably, according to the invention, the organic salt is alverine citrate and the mineral salt is alverine chloride.

It is also possible to use manganese, whether in ionic form or in salt form or in the form of natural, plant or microorganism extracts, particularly bacterial extracts, which are rich in manganese.

Organic manganese salts that may be mentioned

include manganese gluconate, manganese carbonate, manganese acetate, manganese citrate, manganese oleate and manganese oxalate.

Mineral manganese salts that may be mentioned include manganese chloride, manganese borate, manganese nitrate, manganese phosphate and manganese sulphate.

Needless to say, if a manganese-rich natural plant or microorganism extract, particularly a bacterial extract, is used, a person skilled in the art knows how to adapt the amount of extract to be used in order finally to use the manganese in amounts that are suitable for the desired effect.

As manganese-rich natural extracts that may be used according to the invention, mention may be made of extracts of walnut or extracts of tea.

A preferred calcium inhibitor that will be used in the context of the invention is magnesium or its salts.

The effects of magnesium, deduced especially from deficiency and overloading observations, are generally antagonistic to those of calcium. Magnesium is known to inhibit cationic channels, sodium channels and especially calcium-receptor and voltage-dependent channels, and behaves as an anti-calcium agent (M.L. Olinger, *Disorders of calcium and magnesium metabolism*, The Emergency Medecine Clinics of North America, Vol. 7, No. 4, November 1989).

Preferred magnesium salts that may be mentioned include magnesium sulphate, magnesium gluconate, magnesium aspartate, magnesium chloride and magnesium pidolate.

The amount of compounds that may be used according to the invention obviously depends on the desired effect, and may thus vary within a wide range.

To give an order of magnitude, the peptide or the peptide mixture may be used in an amount representing from 0.000001% to 1% of the total weight of the composition and preferably in an amount representing from 0.00001% to 0.01% of the total weight of the composition.

Similarly, it is possible to use according to the invention a calcium-channel inhibitor in an amount representing from 0.0001% to 10% of the total weight of the composition and preferably in an amount representing from 0.01% to 1% of the total weight of the composition.

The composition according to the invention is suitable for topical application to the skin and thus contains a physiologically acceptable medium, i.e. a medium that is compatible with the skin and optionally with its integuments (eyelashes, nails and hair) and/or mucous membranes.

This composition may be in any presentation form normally used in cosmetics, and it may especially

be in the form of an optionally gelled aqueous solution, a dispersion of the lotion type, optionally a two-phase lotion, an emulsion obtained by dispersing a fatty phase in an aqueous phase (O/W emulsion) or conversely (W/O emulsion), or a triple emulsion (W/O/W or O/W/O emulsion) or a vesicular dispersion of ionic and/or nonionic type. These compositions are prepared according to the usual methods. A composition in the form of an oil-in-water emulsion is preferably used according to this invention.

This composition may be more or less fluid and may have the appearance of a white or coloured cream, an ointment, a milk, a lotion, a serum, a paste or a mousse. It may optionally be applied in the form of an aerosol. It may also be in solid form, in particular in the form of a stick. It may be used as a care product and/or as a makeup product for the skin.

In a known manner, the composition used according to the invention may also contain adjuvants that are common in cosmetics, such as hydrophilic or lipophilic gelling agents, hydrophilic or lipophilic active agents, preserving agents, antioxidants, solvents, fragrances, fillers, screening agents, pigments, odour absorbers and dyestuffs. The amounts of these various adjuvants are those conventionally used in the field under consideration, and, for example, from 0.01% to 20% relative to the total weight of the

composition. Depending on their nature, these adjuvants may be introduced into the fatty phase, into the aqueous phase, or into lipid vesicles. In any case, these adjuvants, and also the proportions thereof, will be chosen so as not to harm the desired properties of the combination of anti-wrinkle active agents according to the invention.

When the composition used according to the invention is an emulsion, the proportion of the fatty phase may range from 5% to 80% by weight and preferably from 5% to 50% by weight relative to the total weight of the composition. The oils, emulsifiers and co-emulsifiers used in the composition in emulsion form are chosen from those conventionally used in the field under consideration. The emulsifier and co-emulsifier are present in the composition in a proportion ranging from 0.3% to 30% by weight and preferably from 0.5% to 20% by weight relative to the total weight of the composition.

As oils which may be used in the invention, mention may be made of mineral oils (liquid petroleum jelly or hydrogenated polyisobutene), oils of plant origin (avocado oil or soybean oil), oils of animal origin (lanolin), silicone oils (cyclomethicone or dimethicone) and fluoro oils (perfluoropolyethers). Fatty alcohols (cetyl alcohol), fatty acids and waxes (carnauba wax or ozokerite) may also be used as fatty

substances.

As examples of emulsifiers and co-emulsifiers that may be used in the invention, mention may be made for example of fatty acid esters of polyethylene glycol such as PEG-100 stearate, and fatty acid esters of glycerol such as glyceryl stearate.

Hydrophilic gelling agents that may be mentioned in particular include carboxyvinyl polymers (carbomer), acrylic copolymers such as acrylate/alkylacrylate copolymers, polyacrylamides, in particular crosslinked polyacrylamido-methylpropane-sulphonic acid, polysaccharides, natural gums and clays, and lipophilic gelling agents that may be mentioned include modified clays, for instance bentones, metal salts of fatty acids, hydrophobic silica and polyethylenes.

As active agents, it will be advantageous to introduce into the composition used according to the invention at least one compound chosen from: desquamating agents; moisturizers; depigmenting or propigmenting agents; anti-glycation agents; NO-synthase inhibitors; agents for stimulating the synthesis of dermal or epidermal macromolecules and/or for preventing their degradation and in particular agents for stimulating the synthesis of epidermal macromolecules, such as an extract of beech buds (especially the product sold by the company Gattefosse

under the trade name Gatuline), agents for stimulating collagen synthesis, such as soybean protein hydrolysates (especially the product sold by the company Coletica under the trade name Phytokine), agents for stimulating elastin synthesis and/or for inhibiting collagen degradation, such as an extract of the alga *Macrocystis pyrifera* (especially the product sold by the company Secma under the trade name Kelpadelie) and agents for stimulating glycosaminoglycan synthesis, such as an extract of *Saccharomyces cerevisiae* (especially the product sold by the company Cognis under the trade name Cytovitin); agents for stimulating fibroblast and/or keratinocyte proliferation or for stimulating keratinocyte differentiation, and in particular a soybean protein extract such as the product sold by the company Cognis under the trade name Eleseryl; muscle relaxants; tensioning agents such as polymers comprising a polysiloxane skeleton onto which are grafted mixed polymer units from the poly(meth)acrylic acid type and of the polyalkyl (meth)acrylate type, and in particular those sold by the company 3M under the trade names LO21 and VS80; antipollution agents and/or free-radical scavengers; agents that act on the capillary circulation; agents that act on the energy metabolism of cells; and mixtures thereof.

The compositions in accordance with the

invention may also comprise at least one UVA-active and/or UVB-active organic and/or mineral photoprotective agent (absorbers), which are water-soluble or liposoluble, or even insoluble in the cosmetic solvents commonly used.

The organic photoprotective agents are chosen especially from anthranilates; cinnamic derivatives; dibenzoylmethane derivatives; salicylic derivatives; camphor derivatives; triazine derivatives such as those described in patent applications US 4 367 390, EP 863 145, EP 517 104, EP 570 838, EP 796 851, EP 775 698, EP 878 469, EP 933 376, EP 507 691, EP 507 692, EP 790 243 and EP 944 624; benzophenone derivatives; β,β,-diphenylacrylate derivatives; benzotriazole derivatives; benzalmalonate derivatives; benzimidazole derivatives; imidazolines; bis-benzazolyl derivatives as described in patents EP 669 323 and US 2 463 264; p-aminobenzoic acid (PABA) derivatives; methylenebis(hydroxyphenylbenzotriazole) derivatives as described in patent applications US 5 237 071, US 5 166 355, GB 2 303 549, DE 197 26 184 and EP 893 119; screening polymers and screening silicones such as those described especially in patent application WO 93/04665; dimers derived from α-alkylstyrene such as those described in patent application DE 198 55 649; 4,4-diarylbutadienes as described in patent applications EP 0 967 200,

DE 197 46 654, DE 197 55 649, EP-A-1 008 586, EP 1 133 980 and EP 133 981, and mixtures thereof.

The organic photoprotective agents that are more particularly preferred are chosen from the following compounds: ethylhexyl salicylate, ethylhexyl methoxycinnamate, octocrylene, phenylbenzimidazole-sulphonic acid, benzophenone-3, benzophenone-4, benzophenone-5, 4-methylbenzylidenecamphor, terephthalylidenedicamphorsulphonic acid, disodiumphenyldibenzimidazoletetrasulphonate, 2,4,6-tris(diisobutyl 4'-aminobenzalmalonate)-s-triazine, anisotriazine, ethylhexyltriazone, diethylhexylbutamidotriazone, methylenebis(benzotriazolyl)tetramethylbutylphenol, drometrizole trisiloxane, 1,1-dicarboxy(2,2'-dimethylpropyl)-4,4-diphenylbutadiene, and mixtures thereof.

The mineral photoprotective agents are chosen from pigments or nanopigments (mean size of the primary particles: generally between 5 nm and 100 nm and preferably between 10 nm and 50 nm) of coated or uncoated metal oxides, for instance nanopigments of titanium oxide (amorphous or crystallized in rutile and/or anatase form), of iron oxide, of zinc oxide, of zirconium oxide or of cerium oxide, which are all UV-photoprotective agents that are well known per se. Standard coating agents are, moreover, alumina and/or

aluminium stearate. Such coated or uncoated metal oxide nanopigments are described in particular in patent applications EP 518 772 and EP 518 773.

The photoprotective agents are generally present in the compositions according to the invention in proportions ranging from 0.1% to 20% by weight relative to the total weight of the composition and preferably ranging from 0.2% to 15% by weight relative to the total weight of the composition.

The invention also relates to the cosmetic use of at least one combination as described above, in a composition suitable for topical application to the skin, as an agent for smoothing out wrinkles and fine lines, in particular expression wrinkles.

A subject of the invention is also a cosmetic process for treating wrinkled skin, comprising the topical application to the said skin of a composition according to the invention, in particular to the areas of the face or forehead marked with expression wrinkles and/or to individuals with expression wrinkles.

According to one particular process of the invention, the composition is applied to the wrinkles and fine lines lying radially around the mouth and/or the eyes and/or horizontally on the forehead and/or in the space between the eyebrows.

The invention will now be illustrated with the non-limiting examples that follow. In these

examples, the amounts are indicated as percentages by weight.

The figure is a histogram representing the effect of magnesium gluconate (10^{-4} M) and of 1% Argireline® on calcium channels and also the effect of their combination on type-L calcium channels, verapamil site.

EXAMPLES

Example 1: Demonstration of an inhibitory effect of Argireline on type-L calcium channels, and of an inhibitory synergistic effect of the Argireline-magnesium combination on type-L calcium channels, verapamil site

The test measures the capacity of a product to competitively inhibit the binding of type-L calcium-channel agonists.

The studies are performed using rat cerebral cortex homogenates (isolated membranes having type-L calcium channels at their surface).

The principle of an equilibrium displacement experiment consists in measuring the specific binding at equilibrium, of a given concentration of radiolabelled ligand in the presence of a variable and increasing concentration of cold ligand. The cold ligand enters into competition with the radioactive ligand for its binding to the receptor; this is why this may be termed binding competition at equilibrium.

This technique makes it possible:

- to demonstrate that a cold ligand binds to a receptor
- to study the binding of a ligand with weak affinity for a receptor.

The test products are listed in Table 1 below:

Table 1

Compound	MW	Stock solution
1% Argireline®		100% (v/v) in water
10^{-4} M magnesium sulphate or gluconate	414.3	1×10^{-2} M in water
Mixture of 10^{-4} M magnesium gluconate and 1% Argireline®	-	1×10^{-3} M in water

The experimental conditions according to the protocol described by Reynolds I.J. et al., 1986, *J. Pharmacol. Exp. Ther.*, 237, p. 731 are presented in Table 2 below:

Table 2

Test	Ligand	Conc.	Non-specific	Incubation	Detection
Ca^{2+} channel (L, verapamil	(^{3}H) (-) D 888	0.5 nM	D 600 (10 µM)	60 min/ 22°C	Scintillation

site) counting

D 888 and D 600 are the reference molecules specific for the type-L calcium channels, verapamil site.

The specific binding of a ligand (labelled D 888) to the receptors (type-L calcium channels, verapamil site) is defined as the difference between the total binding and the specific binding determined in the presence of an excess of cold ligand. The results are expressed as a percentage of specific binding and as a percentage of inhibition of specific binding in the presence of the test compounds (Argireline®).

The results obtained for the verapamil site are given in Table 3 below and in the attached figure. The mean percentage of binding (%) and the mean percentages of inhibition are indicated.

Table 3

Compounds	Concen-tration	% of binding	Mean % of binding	Mean % of inhibition
Argireline®	1.0%	95.1 93.1 99.9	96.1	4
Magnesium gluconate	1×10^{-4} M	93.8 95.2 93.0	94.0	6
Magnesium gluconate/ Argireline®	1×10^{-4} M/ 1.0%	81.6 85.3 85.1	84.0	16

The % of binding corresponds to the percentage of binding of the ligand in the presence of Argireline®, which acts as the competitor at the verapamil site.

These results thus show that Argireline® inhibits type-L calcium channels, verapamil site. The effect is observed mainly at the highest two concentrations, 5% and 10%, with mean percentages of inhibition of 36% and 52%, respectively. At a concentration of 1%, Argireline has a moderate effect (4%).

Magnesium gluconate used alone at concentrations of 1×10^{-6} M to 1×10^{-4} M has moderate effects on the type-L calcium channels, verapamil site. At the highest concentration, the mean percentage of inhibition is 6%.

Surprisingly, it is noted that the 1% Argireline®/10^{-4} M magnesium gluconate combination has an inhibitory synergistic effect on type-L calcium channels, verapamil site. The effect represents a mean inhibition of 16%, i.e. an effect greater than the sum of the effects of each of the compounds.

Example 2: Formulation examples

The following compositions are prepared in a conventional manner for those skilled in the art. The amounts indicated are percentages by weight.

COMPOSITION 1: O/W EMULSION

Phase A	Water	qs 100%
	Preserving agents	0.35%
	Manganese gluconate	0.05%
Phase B1	Silicone oils	3%
	Cetyl alcohol and stearyl alcohol	1.6%
	Hydrogenated polyisobutene	4%
	Polyethylene glycol (100 EO)	2%
Phase B2	Volatile silicone oils	3%
Phase D	Crosslinked polyacrylamido methylpropanesulphonic acid (Hostacerin AMPS from Clariant)	1%
Phase E	Acetyl glutamyl-glutamyl-methionyl-glutaminyl-arginyl-arginylamide hexapeptide at 0.05% in water (Argireline from Lipotec)	1%

COMPOSITION 2: W/O EMULSION

Phase A	Water	qs 100%
	Citric acid	0.05%
	Preserving agents	0.95%
	50% sodium hydroxide	2.98%
	Vinylpyrrolidone/styrene copolymer as a 40% emulsion	3.34%
	Fucose-rich polysaccharide at 1% in water (Fucogel from Solabia)	2%
	Oxyethylenated (20 EO) sorbitan	1%

	monolaurate	
	Sodium citrate	1.6%
	Magnesium gluconate	0.05%
	Acetyl glutamyl-glutamyl-methionyl-glutaminyl-arginyl-arginylamide hexapeptide at 0.05% in water (Argireline from Lipotec)	1%
Phase B	Oxyethylenated (18 EO) oxypropylenated (18 PO) mixture of cyclopentasiloxane and polydimethylsiloxane	10%
	Cyclopentasiloxane	8%
	Siloxane elastomer at 20% in polydimethylsiloxane (KSG-1 from Shin-Etsu)	3%
Phase C	Acrylamide/sodium acrylamido-2-methylpropanesulphonate copolymer as a 40% inverse emulsion (Sepigel 305 from SEPPIC)	2%

COMPOSITION 3: GEL

Glycerol	4%
Propylene glycol	3%
Preserving agents	qs
Acrylamide/sodium acrylamido-2-methylpropanesulphonate copolymer as a 40% inverse emulsion	1.5%

(Sepigel 305 from SEPPIC)	
Methacrylate copolymer powder	1%
Alverine	0.1%
Acetyl glutamyl-glutamyl-methionyl-glutaminyl-arginyl-arginylamide hexapeptide at 0.05% in water (Argireline from Lipotec)	4%
Water	qs 100%

COMPOSITION 4: SERUM

Sodium hydroxide	0.1%
Tocopheryl acetate	0.5%
Disodium salt of EDTA	0.1%
Hydrogenated polyisobutene	4.0%
Caffeine	0.2%
Magnesium gluconate	0.5%
Glycerol	3.0%
Preserving agents	1.2%
Cyclohexadimethylsiloxane	6.0%
Crosslinked polyacrylamidomethyl-propanesulphonic acid (Hostacerin AMPS from Clariant)	1.0%
Yeast extract	0.3%
Acetyl glutamyl-glutamyl-methionyl-glutaminyl-arginyl-arginylamide hexapeptide at 0.05% in water (Argireline from Lipotec)	1.0%

Citric acid	0.1%
Tensioning agents	7.0%
Water	qs 100%

In each of the preceding formulations, the Argireline® may be replaced with one of the other peptides that may be used according to the invention.

The above compositions are intended to be applied to the face in the morning and/or in the evening to correct expression wrinkles and fine lines and to relax the marks.

CLAIMS

1. Composition, suitable for topical application to the skin, comprising, in a physiologically acceptable medium, (i) at least one peptide or mixture of peptides comprising an amino acid sequence derived from the amino acid sequence of the protein SNAP 25, and (ii) at least one calcium-channel inhibitor.

2. Composition according to Claim 1, in which the peptide is the hexapeptide defined by SEQ ID No. 2.

3. Composition according to either of Claims 1 and 2, in which the peptide is chosen from:

(i) a peptide that is "substantially homologous" with the peptide defined by SEQ ID No. 2;

(ii) a peptide that is "functionally equivalent" to the peptide defined by SEQ ID No. 2;

(iii) a cosmetically acceptable salt of the said peptide defined by SEQ ID No. 2;

(iv) a peptide defined by SEQ ID No. 2 that has undergone reversible chemical changes.

4. Composition according to Claim 1, in which the peptide or the peptide mixture is chosen from:

a. a peptide comprising a sequence of 3 to 30 amino acids contained in SEQ ID No. 1;

b. a peptide comprising a sequence of 6 to 19

amino acids derived from the N-terminus of the protein SNAP 25, chosen from the group formed by the peptides defined by SEQ ID No. 2 and SEQ ID No. 3;

c. a peptide comprising a sequence of 6 to 19 amino acids derived from the C-terminus of the protein SNAP 25, chosen from the group formed by the peptides defined by SEQ ID No. 5 and SEQ ID No. 6;

d. a peptide mixture consisting of at least one peptide of 3 to 30 amino acids from those described in a), b) and c) and of at least one peptide of 3 to 30 amino acids contained in SEQ ID No. 4;

e. a peptide mixture consisting of at least one peptide chosen from the group formed by the peptides defined by SEQ ID No. 2 and SEQ ID No. 3 and of at least one peptide chosen from the group formed by the peptides defined by SEQ ID No. 5 and SEQ ID No. 6.

5. Composition according to one of Claims 1 to 4, characterized in that the calcium-channel inhibitor is chosen from alverine and/or its salts, manganese and/or its salts, and magnesium and/or its salts.

6. Composition according to one of Claims 1 to 5, characterized in that the calcium-channel

inhibitor is magnesium gluconate.

7. Composition according to one of Claims 1 to 6, characterized in that it comprises at least the hexapeptide defined by the sequence SEQ ID No. 2 and at least magnesium gluconate.

8. Composition according to one of Claims 1 to 7, characterized in that the said peptide or peptide mixture represents from 0.000001% to 1% of the total weight of the composition.

9. Composition according to one of Claims 1 to 8, characterized in that the said peptide or peptide mixture represents from 0.00001% to 0.01% of the total weight of the composition.

10. Composition according to one of Claims 1 to 9, characterized in that the calcium-channel inhibitor represents from 0.0001% to 10% of the total weight of the composition.

11. Composition according to any one of Claims 1 to 10, characterized in that the calcium-channel inhibitor represents from 0.01% to 1% of the total weight of the composition.

12. Composition according to any one of Claims 1 to 11, characterized in that the said composition also contains at least one active agent chosen from: desquamating agents; moisturizers; depigmenting or propigmenting agents; anti-glycation agents; NO-synthase inhibitors; agents for stimulating

the synthesis of dermal or epidermal macromolecules and/or for preventing their degradation; agents for stimulating fibroblast and/or keratinocyte proliferation or for stimulating keratinocyte differentiation; muscle relaxants; tensioning agents; antipollution agents and/or free-radical scavengers; agents that act on the capillary circulation; agents that act on the energy metabolism of cells; and mixtures thereof.

13. Cosmetic use (i) of at least one peptide or peptide mixture comprising an amino acid sequence derived from the amino acid sequence of the protein SNAP 25, and (ii) at least one calcium-channel inhibitor, according to one of the preceding claims, in a composition suitable for topical application to the skin, as an agent for preventing or treating, in particular for smoothing out, wrinkles and fine lines.

14. Use according to Claim 13, characterized in that the said wrinkles are expression wrinkles.

15. Cosmetic process for treating wrinkled skin, comprising the topical application to the said skin of a composition according to any one of Claims 1 to 12.

16. Process according to Claim 15, characterized in that the said composition is applied to the areas of the face or forehead marked with expression wrinkles and fine lines and/or to

individuals with expression wrinkles and fine lines.

17. Process according to either of Claims 15 and 16, characterized in that the said composition is applied to the wrinkles and fine lines lying radially around the mouth and/or the eyes and/or horizontally on the forehead and/or in the space between the eyebrows.

ABSTRACT

USE OF A COMBINATION OF COMPONENTS WITH AN INHIBITORY SYNERGISTIC EFFECT ON CALCIUM CHANNELS TO PREVENT OR TREAT WRINKLES AND FINE LINES

The invention relates to a composition that is suitable for topical application to the skin, comprising, in a physiologically acceptable medium (i) at least one peptide or a mixture of peptides comprising an amino acid sequence derived from the amino acid sequence of the protein SNAP 25, and (ii) at least one calcium-channel inhibitor.

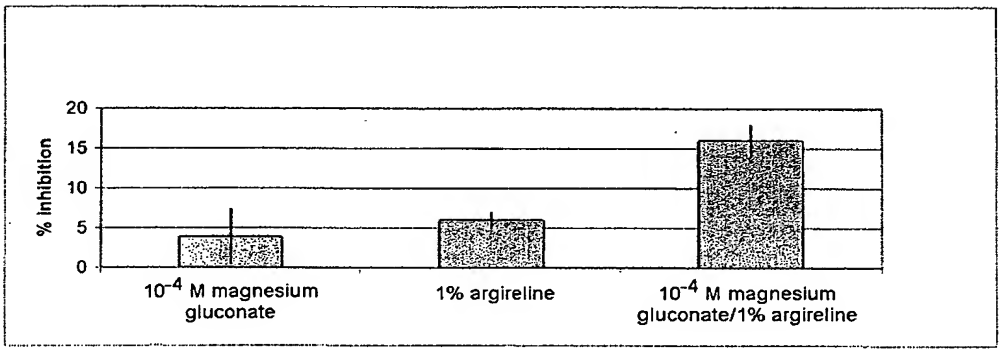
The invention also relates to the use of this combination in a composition suitable for topical application to the skin, as an agent for preventing or treating wrinkles and fine lines, in particular expression wrinkles, and also to a cosmetic treatment process comprising the application of the said composition to the skin.

60427575.112002

APPLN. FILING DATE: NOVEMBER 20, 2002
TITLE: USE OF A COMBINATION OF COMPONENTS WITH AN INHIBITORY SYNERGISTIC EFFECT ON CALCIUM CHANNELS TO PREVENT OR TREAT WRINKLES AND FINE LINES
INVENTOR(S): NONE AT THIS TIME
APPLICATION SERIAL NO:

SHEET 1 OF 1

1/1



Single figure

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